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Supplemental Material to:

Claudia Rentsch, Wolfgang Schneiders, Suzanne Manthey, Barbe Rentsch, and Stephan Rammelt

Comprehensive histological evaluation of bone implants

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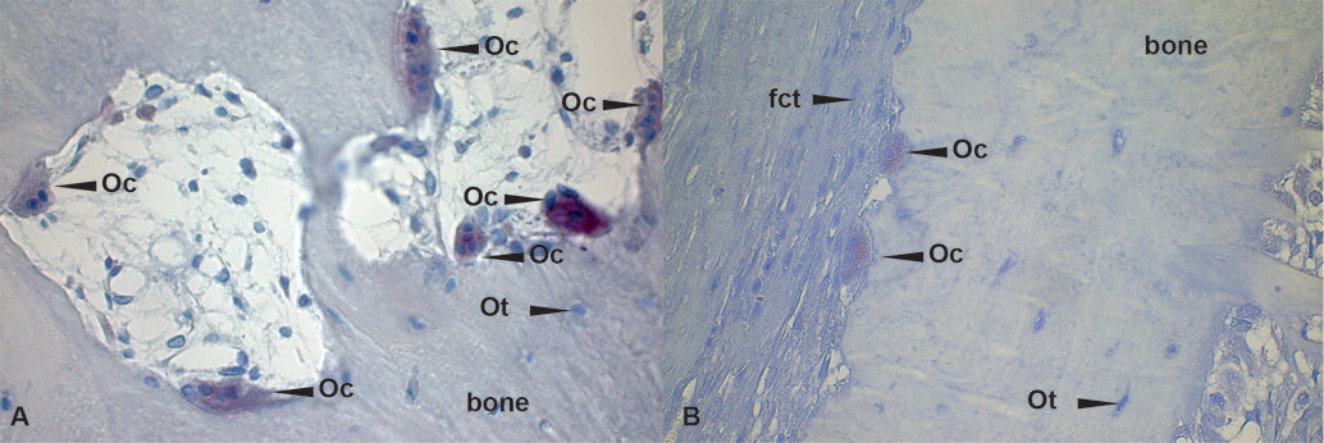
Oc
Oc
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A
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fct
Oc
Oc
Ot
B

Histology/Immunohistology

The time of sample fixation may play a role for immunohistological staining procedures. The fixation time depends on the sample size and the fixative. Formalin penetration is slow (approximately 0.5mm/hour) and under fixation is a much greater problem than over fixation. A meanly fixed bone sample provides no information since cell structures decay. In contrast, over fixation can destroy protein structures and enzyme activity. Therefore, the time of fixation plays a relevant role for immunohistological quality. In this publication 7 days of fixation for large bone samples worked well for histology and immunohistology. Investigations about different fixation time points (data not shown) revealed that e.g. tartrate resistant acidic phosphatase (TRAP) staining is reduced after 8 weeks of formalin fixation (see Figure S1), but not before.

Figure S1. TRAP staining. (**A**) TRAP staining after 7 days of fixation showed a distinct red stain of the osteoclasts. (**B**) TRAP staining after 8 weeks of fixation showed a reduced red stain of the osteoclasts. (original magnification 400x)

Oc: osteoclast, Ot: osteocyte, fct: firm connective tissue